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Nucleotide Metabolism in Salt-Stressed *Zea mays* L. Root Tips

I. ADENINE AND URIDINE NUCLEOTIDES

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ABSTRACT

Corn plants (*Zea mays* L. cv Pioneer 3906) were grown in a glass house on control and saline nutrient solutions, in winter and summer. There were two saline treatments, both with osmotic potential = −0.4 megapascal but with different Ca^{2+}/Na^{+} ratios: 0.03 and 0.73. Root tips and shoot meristems (culm tissue) of 26 day-old plants were analyzed for nucleotides to ascertain if there were correlations between nucleotide pool size and the reduced growth on saline cultures. Several other cell components also were determined. Plants grown in winter were only half as large as those grown in summer mainly because of the lower light intensity and lower temperature. But the relative yield reduction on salt treatment compared to the control was similar in winter and summer. The two different salt treatments caused similar yield reductions. Neither salt treatment affected nucleotide pools in culm tissue, with the possible exception of UDPG in winter. In the case of root tips, salt treatment had little or no effect on nucleotide pool sizes in winter when many already seemed near a critical minimum, but in summer it reduced several pools including ATP, total adenine nucleotide, UTP, total uridine nucleotide, and UDP-glucose. The reductions were greatest on the salt treatment with low Ca^{2+}/Na^{+}. There was no simple correlation between the effects of salt stress on growth and on nucleotide pool size. The nucleotide pools of culm tissue indicated that in some respects this tissue was effectively insulated from the salt stress. Roots that were in direct contact with the saline solution indicated significant reductions in nucleotide pools only in the summer whereas growth was reduced both summer and winter. It is possible that the nucleotide concentrations of root cells in winter were already near a critical minimum so that nucleotide synthesis and growth were tightly linked. Significant reductions in nucleotide pools that would be expected to affect growth were more evident in summer when pools were larger and growth was more rapid. But even where ATP and total adenine nucleotides were reduced, the ratio of ATP:ADP and the adenylate energy charge remained unchanged indicating an active adenylate kinase that had access to most of the adenine nucleotide pools, and possible catabolism of excess AMP.

Salinity reduces the growth and yield of many important crop species (5, 11, 16), and in severe cases causes crop failure. How salinity affects plant growth is not known, but it is believed to be by a combination of osmotic (water deficit) and ionic effects (5, 11), with the osmotic effect predominating in most cases. One early hypothesis (4) proposed that the growth-limiting process was the active uptake by enlarging cells of the solutes necessary for osmotic water uptake and turgor maintenance. With minor modification and elaboration, this hypothesis is still employed (12). However, some recent evidence (19) seems to indicate that shoot growth of salt-stressed plants, which is usually reduced more than root growth, is not limited by turgor or turgor generating processes. These authors postulate that shoot growth is regulated by a 'message from the root' which is affected by salt stress. The message is not identified, but it could be a hormone or metabolite (19).

One of the first root responses to saline solutions is a marked increase in respiration (3) that is believed to be a result of increased demand for energy, derived from the oxidation of carbohydrates, needed for uptake and compartmentation of ions. This hypothesis is supported by the reduced ATP pool size observed in excised corn root tips shortly after their exposure to salt stress (24). The reduced ATP was preceded by a large increase in cytoplasmic Pi. Both of these changes would reduce the phosphate potential, [ATP]/[ADP]·[Pi], which has been shown to be an important regulator of cell metabolism (8, 10). UDPG also was markedly reduced in the salt-stressed corn root tips, possibly a consequence of the reduced ATP pool and/or the reduced phosphate potential. All of these effects could be consequences of increased ATP expenditures for salt pumping and all could contribute to reduced growth. The objective of these experiments was to ascertain whether the reduction in growth of intact corn plants caused by extended culture on a saline root medium is correlated with reductions in the size of nucleotide pools, especially of AdN,[1] UdN, and UDPG, in root and shoot meristems. The AdN pools are of interest because of their essential role in energy metabolism, transport processes, and metabolic regulation; the UdN and UDPG pools, because they are among the largest pools in plant tissues (18), and UDPG, which is centrally located in glycoside metabolism, was markedly reduced in corn root tips by salt stress (24). Two salt treatments were employed, both with the same Ψ_s (−0.4 MPa) but with different Ca^{2+}/Na^{+} ratios. This ratio was varied in order to investigate the role in salt stress of a sodium-induced calcium deficiency to which corn is susceptible (17).

MATERIALS AND METHODS

Plant Material. Seeds of *Zea mays* L. cv Pioneer 3906, supplied through the courtesy of Pioneer Hi-Bred International, Inc.,[2] were soaked for 30 h in vigorously aerated distilled water, then spread on paper moistened with 0.25 mM $CaSO_4$ and germinated in the dark at room temperature. Three-d-old seedlings were placed on cheesecloth supported between 2 plastic

[1] Abbreviations: AdN, adenine nucleotide; AEC, adenylate energy charge; ΣAdN, sum of AMP, ADP, and ATP; K, mass action ratio for adenylate kinase; UdN, uridine nucleotide; ΣUdN, sum of UMP, UDP, and UTP; UDPG, UDP-glucose; Ψ_s, osmotic potential.

[2] Company and trade names are shown for benefit of the reader and do not imply endorsement or preferential treatment by the United States Department of Agriculture of the company or products noted.

Table I. *Effects of Salt Stress on Weight of Shoots, Roots, Root Tips, and Culm Sections of 26-d-Old Corn Plants*

Means with standard errors in parentheses; means in a row followed by a common letter do not differ significantly ($P \leq 0.05$). Each treatment was replicated three times in winter, four in summer. Each root tip sample contained >50 tips, each culm sample, 20 culm sections.

Quantity and Tissue	Treatment								
	Control			−0.4 MPa NaCl			−0.4 MPa NaCl + $CaCl_2$		
Fresh wt									
Winter shoot, g·plant^{-1}	22.2	(0.8)	a	12.5	(0.3)	b	11.7	(0.8)	b
Summer shoot, g·plant^{-1}	43.2	(0.8)	a	25.3	(1.6)	b	24.1	(0.8)	b
Summer root, g·plant^{-1}	10.1	(0.4)	a	6.6	(0.7)	b	6.5	(0.3)	b
Summer root tip, mg·tip^{-1}	7.61	(0.3)	a	6.8	(0.3)	a	*		
Summer culm, g·culm^{-1}	1.96	(0.1)	a	1.63	(0.1)	b	1.44	(0.1)	b
Dry wt									
Winter shoot, g·plant^{-1}	1.6	(0.1)	a	0.98	(0.03)	b	1.07	(0.04)	b
Summer shoot, g·plant^{-1}	2.91	(0.05)	a	1.63	(0.11)	b	2.03	(0.12)	b
Summer root, g·plant^{-1}	0.46	(0.02)	a	0.33	(0.00)	b	0.41	(0.04)	ab
Summer culm, g·culm^{-1}	0.149	(0.005)	a	0.134	(0.005)	b	0.125	(0.004)	b

* No data.

Table II. *Effects of Salt Stress on the Weight of Cell Wall Fraction and Protein of Root Tip and Culm Samples*

Means with significance and replication as in Table I. Each root tip sample contained 130 root tips, each culm sample, 12 culm sections from 26-d-old plants.

Component and Tissue	Treatment								
	Control			−0.4 MPa NaCl			−0.4 MPa NaCl + $CaCl_2$		
	mg/sample								
Wall Fraction									
Winter root tip	40.6	(2.6)	a	31.0	(1.5)	b	48.3	(5.6)	a
Summer root tip	48.4	(2.6)	a	35.9	(6.4)	a	47.5	(1.1)	a
Winter culm	512.6	(119.4)	a	372.3	(16.8)	a	334.3	(5.8)	a
Summer culm	857.6	(112.9)	a	886.5	(65.8)	a	1026.0	(102.0)	a
Protein									
Winter root tip	10.9	(0.16)	a	9.9	(0.7)	a	11.8	(1.7)	a
Summer root tip	14.8	(1.2)	ac	11.6	(0.5)	a	14.0	(0.6)	bc
Winter culm	48.2	(10.3)	a	33.7	(2.9)	a	35.9	(2.6)	a
Summer culm	21.2	(1.9)	a	27.9	(0.8)	b	22.2	(1.5)	a

grids with 1.7 cm^2 openings. The seedlings, separated by the grid partitions, were covered with moist vermiculite. The grid assemblies were transferred to the glasshouse and supported over plastic pots containing 28 L of aerated nutrient solution. The composition of the nutrient solution was: 2.5 mM $Ca(NO_3)_2$, 3 mM KNO_3, 1.5 mM $MgSO_4$, 0.17 mM KH_2PO_4, 50 μM Fe (as sodium ferric diethylenetriamine pentaacetate), 23 μM H_3BO_3, 5 μM $MnSO_4$, 0.4 μM $ZnSO_4$, 0.2 μM $CuSO_4$, and 0.1 μM H_2MoO_4.

Plants were thinned to 35 seedlings per pot. Seven d after germination the cultures were salinized at a rate calculated to reduce the Ψ_s of the solutions by 0.1 MPa d^{-1}. Three treatments included a nonsaline control and two saline treatments, both of the latter with $\Psi_s = -0.4$ MPa but with different molar ratios of Ca^{2+}/Na^+:0.03 (2.5 mM $CaCl_2$ + 86.5 mM NaCl) and 0.73 (31.5 mM $CaCl_2$ + 43.1 mM NaCl). These treatments are identical to those of an earlier study on sodium-induced calcium deficiency in salt stressed corn (17). Each treatment was replicated three (winter culture) or four (summer culture) times. The pH of the culture solutions was adjusted to pH 5.0, with H_2SO_4, whenever it rose to pH 6.5. All solutions were changed four times during each experimental period. Two separate experiments were conducted with the first set of plants grown during January-February 1985 (Winter Plants) when the average daily maximum/minimum temperatures in the glasshouse were 30.6/19.7°C. The second set of plants was grown during June–July 1985 (Summer Plants) when the average daily maximum/minimum temperatures in the glasshouse were 37.3/22.6°C. The winter and summer average total daily photosynthetically active radiation differed significantly ($P \leq 0.05$) with values of 23.9 ± 1.0 (SE) mol m^{-2} for winter and 46.7 ± 0.6 mol m^{-2} for summer plants. Relative humidity also differed significantly ($P \leq 0.05$) between winter and summer. Maximum/minimum daily averages were 57.7 ± 1.7/36.6 ± 1.2% RH for winter, and 68.7 ± 0.6/37.1 ± 0.7% RH for summer.

Plant Sample Weights. Because analyses for nucleotides required that tissues be quickly harvested and frozen, additional culm samples (2.54 cm basal shoot section, $n = 20$ per treatment) and root tips (5 mm in length, $n > 50$ each for control and sodium chloride treatments) were taken for fresh and dry weight determinations. Wall fraction dry weights of root tip and culm samples were obtained as part of the sample preparation for the protein assay described below.

Nucleotide Extraction. Twenty-six d after germination, 16 d on full salt treatment, 5 mm root tips and 2.54 cm culm sections, including the intercalary meristem and adjacent tissues, were collected for nucleotide extraction. One-hundred and thirty root

Table III. *Effects of Salt Stress on Concentrations of Pi, Acid-Soluble Phosphate-Ester, Total Hexose and Sucrose, in Root Tips and Culm Sections*

Means with significance and replication as in Table I. Sample size as in Table II. Note the different units for root and culms.

Tissue and Substance	Treatment								
	Control			−0.4 MPa NaCl			−0.4 MPa NaCl + $CaCl_2$		
	nmol/root tip								
Winter root tip									
Pi	33.7	(1.9)	a	29.3	(3.8)	a	28.0	(2.3)	a
Pe[a]	10.0	(1.0)	a	4.7	(1.3)	b	11.3	(3.8)	a
Total hexose	417.3	(42.9)	a	317.0	(29.9)	a	540.7	(25.5)	b
Sucrose	11.2	(2.2)	a	4.7	(0.4)	a	4.2	(0.1)	a
Summer root tip									
Pi	25.9	(3.2)	a	41.3	(10.2)	ab	49.8	(3.3)	b
Pe	28.3	(1.5)	a	23.3	(2.8)	a	29.1	(0.6)	a
Total hexose	928.0	(61.7)	a	633.8	(56.9)	b	791.1	(67.0)	ab
Sucrose	508.5	(63.7)	a	166.5	(56.7)	b	443.2	(34.4)	a
	μmol/culm								
Winter culm									
Pi	1.5	(0.2)	a	4.5	(0.4)	b	0.6	(0.1)	c
Pe	0.93	(0.09)	a	1.19	(0.16)	a	0.45	(0.08)	b
Total hexose	0.085	(0.006)	a	0.055	(0.005)	b	0.062	(0.008)	ab
Sucrose	0.014	(0.00)	a	0.008	(0.000)	b	0.004	(0.000)	c
Summer culm									
Pi	0.51	(0.07)	a	0.99	(0.16)	b	1.02	(0.08)	b
Pe	0.99	(0.37)	a	0.77	(0.06)	a	0.94	(0.18)	a
Total hexose	161.1	(20.8)	a	97.7	(18.1)	b	88.3	(5.1)	b
Sucrose	6.9	(1.0)	a	6.6	(1.2)	a	5.7	(0.7)	a

[a] Acid-soluble phosphate-ester.

Table IV. *Effects of Salt Stress on the Total Mononucleotide Content of Root Tips and Culm Sections*

Total mononucleotide = the sum of AMP, ADP, ATP, GMP, GDP, GTP, CMP, CDP, CTP, UMP, UDP, UDP, UTP. Means with significance and replication as in Table I. Sample size as in Table II.

Tissue	Treatment								
	Control			0.4 MPa NaCl			−0.4 MPa NaCl + $CaCl_2$		
	nmol/root tip								
Winter root tip	10.8	(1.6)	a	8.6	(0.2)	a	10.0	(0.7)	a
Summer root tip	13.2	(1.4)	a	7.55	(0.5)	b	10.5	(0.4)	a
	nmol/culm								
Winter culm	505.7	(63.5)	a	366.1	(83.3)	a	328.2	(19.4)	a
Summer culm	452.6	(58.8)	a	560.6	(80.1)	a	673.4	(79.4)	a

tips and 12 culm sections were harvested from each culture and immediately frozen in liquid N_2. The root tips or culms from a given culture were composited to make one sample. The remaining shoots and roots from each culture were harvested for plant yield data.

Nucleotides were extracted from frozen tissues using cold perchloric acid following the protocol of Nieman and Clark (22) and separated from other compounds and ions by selective adsorption (21). Chromatographic separation and determination of nucleotides was achieved by anion exchange HPLC using a phosphate buffer gradient (22). Nucleotide peak purity and identity were confirmed by comparison with standards and spectral analysis, between 190 and 300 nm, using a Hewlett Packard 1040A Diode Array Detector.

Protein, Sugar, and Wall Content. The acid insoluble pellet left after nucleotide extraction was washed with 80% ethanol, dried at 37°C overnight, and weighed as an estimate of cell wall material. The material was then suspended with 0.3 M NaOH and incubated overnight at 37°C. The NaOH extract was used for protein determinations using the Coomassie brilliant blue colorimetric assay (Bio-Rad) with bovine serum as the standard.

The 0.01 M HCl eluate from Amberlite XAD-2, polyvinylpolypyrrolidone, and charcoal columns, after nucleotide isolation (21), was saved for the determination of Pi (26), total acid-soluble P (2), total hexose (25), and sucrose (13). Organic ester phosphate (Pe) was estimated as the difference between total acid-soluble P and Pi.

Statistical Analysis. Analysis of variance and Student's *t*-test were used to test the significance of treatment effects in Tables I through VII, and weather data. Values were accepted as significantly different at $P \leq 0.05$.

RESULTS

Plant Yield, Protein, and Wall Content. The yield data (Table I) show that despite a strong seasonal effect, where fresh yield was twice as large in the summer—and the sugar content was orders of magnitude greater (Table III), both salt treatments reduced yield by the same relative amount, about half, in summer

Table V. *Effects of Salt Stress on Adenine Nucleotide Pool Sizes and Ratios in Root Tips and Culm Sections*
Means with significance and replication as in Table I. Sample size as in Table II.

Tissue and Nucleotide	Treatment								
	Control			−0.4 MPa NaCl			−0.4 MPa NaCl + $CaCl_2$		
	nmol/root tip								
Winter root tip									
AMP	0.18	(0.02)	ab	0.11	(0.02)	a	0.22	(0.01)	b
ADP	0.55	(0.04)	ac	0.37	(0.02)	b	0.46	(0.02)	c
ATP	1.54	(0.15)	a	1.31	(0.13)	a	1.47	(0.11)	a
ΣAdN	2.3	(0.4)	a	1.8	(0.1)	a	2.15	(0.1)	a
ATP/ADP	2.73	(0.20)	a	3.50	(0.12)	a	3.16	(0.11)	a
AEC*	0.79	(0.01)	ab	0.83	(0.01)	a	0.79	(0.00)	b
K**	0.92	(0.13)	a	1.05	(0.14)	a	1.53	(0.09)	b
Summer root tip									
AMP	0.22	(0.04)	ab	0.13	(0.02)	a	0.232	(0.02)	b
ADP	0.81	(0.08)	a	0.55	(0.07)	a	0.73	(0.07)	a
ATP	2.13	(0.06)	a	1.36	(0.08)	b	1.61	(0.07)	c
ΣAdN	3.2	(0.2)	a	2.0	(0.2)	b	2.6	(0.1)	ab
ATP/ADP	2.72	(0.24)	a	2.57	(0.19)	a	2.25	(0.18)	a
AEC	0.81	(0.02)	a	0.81	(0.01)	a	0.77	(0.03)	a
K	0.71	(0.06)	a	0.59	(0.03)	a	0.67	(0.03)	a
	nmol/culm								
Winter culm									
ΣAdN	136.2	(15.7)	a	96.2	(19.0)	a	87.6	(14.5)	a
ATP/ADP	2.81	(0.61)	a	2.26	(0.60)	a	3.30	(0.76)	a
AEC	0.77	(0.03)	a	0.73	(0.02)	a	0.77	(0.04)	a
K	1.31	(0.27)	a	1.40	(0.64)	a	1.77	(0.42)	a
Summer culm									
ΣAdN	112.2	(12.7)	a	115.6	(14.7)	a	125.4	(11.7)	a
ATP/ADP	2.23	(0.03)	a	1.95	(0.10)	a	1.90	(0.06)	a
AEC	0.74	(0.04)	a	0.69	(0.03)	a	0.71	(0.28)	a
K	0.93	(0.12)	a	1.15	(0.18)	a	0.89	(0.11)	a

* AEC = ([ATP] + ½ [ADP])/([AMP] + [ADP] + [ATP]). ** K = (ATP × AMP)/ADP^2.

as well as in winter. Season had little effect on wall fraction and protein of root tips (Table II) but it did influence these quantities in culms. In summer, culm sections had more cell wall material but less protein (Table II). The tissue samples used for nucleotide extraction were not weighed because of the necessity for rapid harvest and freezing. For that reason, the concentrations of cell constituents are reported on a root tip or culm basis. Additional samples were harvested for weight data so that quantities may be converted to a weight basis. The data (Tables I and II) show that salt stress did not significantly affect the weight of root tips, their wall fraction—except for the NaCl treatment in winter, or their protein content. Cell counts (TA Peterson, CJ Lovatt, RH Nieman, unpublished data) also showed that salt treatment did not significantly change the number of cells per root tip. Because the root tip weights tended to remain fairly constant regardless of treatment, quantities may be converted from a root tip basis to a g fresh or dry weight basis, respectively, by multiplying by 140 root tips/g fresh weight, or by 3000 root tips/g dry weight.

Culm data are given to provide a comparison of salt effects on shoot meristems with those on root tips. Salt stress reduced the fresh and dry weights of culm sections (Table I), but not their cell wall or protein content (Table II). Because of the changes in weight with treatment, different factors are needed for control and salt-stressed plants to convert quantities from a culm to a weight basis. For example, to convert to a fresh weight basis, the amount per culm is multiplied by 0.5 culm/g fresh weight for controls and by 0.65 culm/g fresh weight for salt treated plants.

Phosphorus, Sugar, and Total Nucleotide Content. Salt treatment had relatively little consistent effect on the acid soluble phosphorus and sugar content of root tips and culms (Table III). Salt effects were conditioned to a considerable extent by the growing season. For example, NaCl treatment reduced root Pe in winter but not in summer; conversely, the high calcium treatment increased root and culm Pi in summer but not in winter.

Roots and culms had a much higher sugar content in the summer, and only then did NaCl significantly reduce root sugar content (Table III). High calcium tended to diminish this effect. In culms, on the other hand, NaCl reduced the sugar content both summer and winter, and high calcium did not diminish this effect.

The only significant effect of either salt treatment on total mononucleotide concentration (the sum of AMP, ADP, ATP, GMP, GDP, GTP, CMP, CDP, CTP, UMP, UDP, UTP) was the reduced concentration in summer roots on the NaCl treatment (Table IV).

Adenine Nucleotide Pools. In root tips, the NaCl treatment tended to reduce all AdN pools (Table V), but the reductions were significant only in the case of ADP in winter and ATP and ΣAdN in summer. High calcium tended to diminish the effect of NaCl, but it did not prevent the significant decrease in ATP of summer roots. Neither salt treatment significantly affected the ATP/ADP ratio or the adenylate energy charge: (AEC = ([ATP] + ½[ADP])/([AMP] + [ADP] + [ATP]). The adenylate kinase mass action ratio: K = ([ATP] × [AMP])/$[ADP]^2$, was unaffected by either salt treatment in summer but was increased by high calcium in winter. In culms, the AdN pools and ratios were not significantly affected by either salt treatment.

Uridine Nucleotide Pools. In winter roots NaCl reduced only UMP (Table VI), but in summer roots it reduced all uridine

Table VI. *Effects of Salt Stress on Uridine Nucleotide and UDPG Pool Sizes in Root Tips and Culm Sections*

Means with significance and replication as in Table I. Sample size as in Table II.

Tissue and Nucleotide	Treatment								
	Control			−0.4 MPa NaCl			−0.4 MPa NaCl + $CaCl_2$		
	nmol/root tip								
Winter root tip									
UMP	0.42	(0.08)	a	0.17	(0.00)	b	0.26	(0.02)	a
UDP	0.53	(0.06)	a	0.41	(0.04)	a	0.49	(0.03)	a
UTP	0.96	(0.07)	a	0.77	(0.08)	a	0.88	(0.09)	a
ΣUdN	1.9	(0.3)	a	1.3	(0.1)	a	1.64	(0.1)	a
UDPG	4.27	(0.18)	a	3.49	(0.28)	a	3.92	(0.21)	a
UTP/UDP	1.80	(0.17)	a	1.90	(0.13)	a	1.77	(0.10)	a
Summer root tip									
UMP	0.49	(0.41)	a	0.29	(0.28)	b	0.71	(0.20)	a
UDP	1.07	(0.30)	a	0.0*		b	0.63	(0.16)	a
UTP	1.08	(0.06)	a	0.63	(0.02)	b	0.83	(0.02)	c
ΣUdN	2.6	(0.3)	a	0.9	(0.1)	b	1.9	(0.4)	ab
UDPG	4.54	(0.32)	a	2.76	(0.24)	b	3.77	(0.30)	c
UTP/UDP	1.00	(0.18)	a	*		b	1.31	(0.28)	a
	nmol/culm								
Winter culm									
ΣUdN	77.7	(12.4)	a	56.2	(7.8)	a	54.0	(3.1)	a
UDPG	48.0	(5.0)	a	51.9	(17.9)	a	39.7	(2.8)	b
UTP/UDP	2.45	(0.63)	a	2.94	(0.40)	a	1.92	(0.35)	a
Summer culm									
ΣUdN	34.9	(3.1)	a	36.7	(5.5)	a	40.9	(6.4)	a
UDPG	97.5	(14.3)	a	112.2	(8.1)	a	124.2	(4.3)	a
UTP/UDP	1.63**			1.74			1.61		

* UDP not detected (<1 nmol/g fresh root). ** Estimates without statistical analysis.

nucleotides including UDPG. It reduced UDP below the limit of detection (<1 nmol/g fresh root). High calcium tended to diminish the effect of NaCl but it did not prevent significant reductions in UTP and UDPG of summer roots. Neither salt treatment affected the individual UdN pools of culms (data not presented) or their ΣUdN or UTP/UDP ratios, but high calcium reduced UDPG in winter culms. The UdN pools did not show the tendency observed with AdN pools to stay in balance regardless of the treatment.

Other Nucleotides. Root tips and culms contained several nucleotides besides AdN and UdN. For the most part, their concentrations were not markedly affected by salt treatment. CMP and GMP frequently were below the limit of detection (<1 nmol/g fresh tissue). In summer roots, however, there were four nucleotides, NAD, GDP, GTP, and CTP, that showed significant reductions in concentration on the NaCl treatment (Table VII). High calcium decreased the NaCl effect on at least two: NAD and GDP.

DISCUSSION

Both salt treatments reduced growth by half in both winter and summer (Table I). Winter grown plants were only half as large as those grown in summer on all treatments. Corn yields commonly are much reduced in off season (6) mainly because of reduced light and temperature. The control plants in winter were about the same size as the salt-stressed plants in summer (Table I), and their AdN pools were about the same size (Table V). Salt stress caused little or no change in AdN pools of winter-grown plants even though it reduced growth by half. AdN pools were larger in control summer-grown plants, but on NaCl treatment they were no larger than in winter-grown controls. The results suggest that there may be a critical minimum AdN pool size in corn roots that will support growth and the pools of winter-grown plants were already near that minimum so that further reductions were closely coupled with reductions in growth.

The size of the individual AdN pools seemed to be regulated so that they tended to remain in balance even where salt stress reduced ΣAdN. This regulation is indicated by the absence of significant treatment effects on the ATP/ADP ratio, the adenylate energy charge (AEC), and, with one exception in winter roots, the adenylate kinase mass action ratio (K) (Table V). These results suggest that an active adenylate kinase had access to essentially all AdN with no significant metabolically inert AdN pools and no significant effect of salt stress on access. The ratios of ATP/ADP and K generally tended to be higher in winter, possibly because with slower growth and less transpiration stress, ATP turnover was slower.

The decrease in ΣAdN of salt-stressed roots with no change in AEC suggests that AMP may have been catabolized, by an AMP deaminase, for example, as a means to prevent a decrease in AEC under conditions of increased ATP hydrolysis (7). Also, salt stress has been shown to increase purine catabolism in corn roots (TA Peterson, CJ Lovatt, RH Nieman, unpublished data). The tendency for AEC to remain constant even under stress means that in the tissues investigated here it is not a sensitive indicator of stress-induced metabolic disturbance.

The UMP, UTP, and UDPG pools of control roots were similar in winter and summer despite the much higher sugar content and more rapid growth in summer. These results indicate a balance between production of these nucleotides and growth. Although salt stress reduced growth in winter it did not significantly affect the size of UDP, UTP, and UDPG pools, so a balance was maintained. Neither did salt stress affect the ratios of UTP/UDP and UDPG/UTP. The latter ratio was constant (4.2–4.5) under all treatment conditions, in winter and summer, despite large differences in sugar content, indicating that the level of UDPG was more closely correlated with the level of UTP

Table VII. *Effects of Salt Stress on the Indicated Nucleotide Pool Sizes in Summer Root Tips*
Means with significance and replication as in Table I. Sample size as in Table II.

Nucleotide	Treatment								
	Control			-0.4 MPa NaCl			−0.4 MPa NaCl + $CaCl_2$		
	nmol/root tip								
NAD	0.63	(0.04)	a	0.39	(0.03)	b	0.56	(0.03)	c
GDP	0.22	(0.01)	a	0.15	(0.01)	b	0.22	(0.01)	a
GTP	0.53	(0.03)	a	0.35	(0.02)	b	0.37	(0.02)	b
CTP	0.53	(0.03)	ac	0.40	(0.02)	b	0.46	(0.02)	bc

than with sugar content. The level of UTP, in turn, was correlated with the level of ATP; the ratio of UTP/ATP for all treatments was 0.6 in winter and 0.5 in summer. In summer, salt stress, especially NaCl, altered the balance between UdN production and growth, reducing UdN and UDPG pools more than growth. Possibly, the higher sugar content in summer allowed nucleotides to turn over more rapidly so that growth proceeded at lower nucleotide levels.

UDPG is essential for sugar metabolism, for cell wall synthesis, and for membrane processing (9). In some cases it may be a limiting factor for wall synthesis (1, 14). UTP pools in turn, might limit the synthesis of UDPG and be limited by ATP pools. Although salt stress did not significantly change the weight of cell wall material per root tip (Table II), it certainly did reduce the total amount of cell wall produced as indicated by the reduced plant dry weight (Table I). The absence of a salt stress effect on the weight of wall material per root tip indicates that wall production and growth remained in balance even where growth was severely reduced by salt stress.

The culm sections were included in this study to provide a comparison of salt stress effects on root and shoot meristems of corn. The nucleotide data (Tables IV, V, and VI) indicate that, with the exception of UDPG in winter, salt stress had no effect on nucleotide pool size in culms. This perhaps is to be expected because culms are more insulated from exposure to salt. The salt concentration is usually low in shoot meristems of nonhalophytes, even when they are grown on saline media (11). Of the major nucleotide pools of culms, only UDPG increased markedly in summer with the enormous increase in sugar (Table VI). ΣUdN decreased, possibly because of greater conversion to UDPG. AdN pools seemed unaffected by the increased sugar.

The dominant effect of salt stress on growth often seems to be osmotic (5, 11), but a number of other effects occur of an ionic nature that undoubtedly contribute to growth reductions (5, 11, 15, 20). In the present experiments, the greater reduction of AdN, UdN, UDPG, and NAD pools of roots caused by the salt treatment with the lower Ca^{2+}/Na^{+} indicates that this effect is at least partly ionic and associated with high sodium. The chloride concentrations were similar (92 and 106 mM) in solutions with low and high Ca^{2+}/Na^{+}. On the other hand, the growth reduction was equivalent with either low or high Ca^{2+}/Na^{+} so this effect seemed to be due more to high total salt or low Ψ_s than to specific ions or cation ratios.

If growth is closely linked to the balance of nucleotide synthesis and catabolism, large changes in the concentrations of nucleotides in tissues would not be expected. But the results presented here indicate that growth was more rapid in tissues with larger nucleotide pools, as in summer controls. Possibly a kinetic study could show more clearly a correlation between growth rate and nucleotide pool size.

Questions regarding the synthesis and turnover of nucleotides within each pool have yet to be resolved. An important beginning has been made with the noninvasive ^{31}P NMR measurements of ATP synthesis and hydrolysis in living tissue (23). Nucleotide pool sizes will be influenced by rates of synthesis and catabolism of purines and pyrimidines. We (TA Peterson, CJ Lovatt, RH Nieman) will present data on the effects of salt stress on the *de novo* synthesis, salvage, and catabolism of these compounds in corn root tips.

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